GateV™ Cloning System Instruction

Overview:

The GateV™ Cloning Expression System by CloneSmarter™ serves as a direct replacement for the Invitrogen™ GateWay™ Cloning System, as its working principle and operation method are identical. The GateV™ cloning and expression system relies on the specific recombination between λ phage and host Escherichia coli to efficiently clone the target gene fragment into the starting vector, resulting in the starting clone. This clone can then be quickly and accurately exchanged and recombined with different expression vectors to obtain expression clones. This method eliminates the cumbersome and inefficient classic enzyme-cut ligation process, significantly simplifying and speeding up the construction process from a single gene to multiple expression vectors. The figure below illustrates the principle of the GateV™ Cloning Expression System:

attB1-gene-attB2 × attP1-ccdB-attP2 ⇔ attL1-gene-attL2 × attR1-ccdB-attR2

The GateV™ Cloning System consists of the following two multi-enzyme premix systems:

I. The GateV™ BP Master Mix is a multi-enzyme system that catalyzes the recombination between the target gene fragments (linear PCR fragments or fragments that have been cloned into vectors) containing attB sequences at both ends and the starting vectors containing attP sequences (such as pDONR™), resulting in the initial clone, also called the Entry clone, which contains attL sequences (sequences formed by the combination of attB and attP sequences after recombination) at both ends.

II. The GateV™ LR Master Mix is another multi-enzyme mixed system that catalyzes the recombination between the target gene containing attL sequences at both ends and the expression vector containing attR sequences, resulting in expression clones.

Components and Storage

1. GateV® BP Master Mix

Components	Cat. No.		Storage Condition
	C6001-10 10 Rxn	C6001-20 20 Rxn	
BP Master Mix	20 ul	40 ul	-20°C for ≤ 6 months, -80°C for long term
Proteinase K	20 ul	40 ul	-20°C
Control	10 ul	10 ul	-20°C

2. GateV® LR Master Mix

Components	Cat. No.		Storage Condition
	C6002-10 10 Rxn	C6002-20 20 Rxn	
LR Master Mix	20 ul	40 ul	-20°C for ≤ 6 months, -80°C for long term
Proteinase K	20 ul	40 ul	-20°C
Control	10 ul	10 ul	-20°C

Instructions and operating steps:

I. GateV™ BP reaction:

1. Thaw the GateV™ BP Master Mix on ice and gently flick the tube several times to mix.
2. At room temperature, add the following reagents to a 1.5 mL centrifuge tube, mix gently, and briefly centrifuge in a centrifuge.

attB-PCR product （~50-150 ng）		1-7 ul
Entry Vector （150 ng/ ul）		1 ul
TE pH8.0	to	8 ul
GateV™ BP Master Mix		2 ul
	Total:	10 ul

At the same time, perform the following controls:

attB1- lacZ –attB2 fragment：	1 ul
Entry vector： pDONOR221 （KanR）	1 ul
TE pH8.0	6 ul
GateV™ BP Master Mix	2 ul
Total:	10 ul

3. Immediately return the GateV™ BP Master Mix to -80°C for long-term storage or to -20°C for short-term storage.
4. Incubate the centrifuge tube at 25°C for 1 hour. For large gene fragments, incubate for 4-6 hours or overnight. The optimal substrate for the BP Master Mix is linear PCR product or linearized plasmid. The efficiency of non-linearized supercoiled plasmids is low.
5. Add 1 μL of Proteinase K and mix well, incubate at 37°C for 10 minutes.
6. Transformation: Take 1-5 μL of the reaction mixture from the centrifuge tube, add it to 50 μL of competent cells, incubate on ice for 20 minutes, heat shock at 42°C for 1 minute, return to ice for 2 minutes, add 250 μL of 2YT or SOC medium, and incubate at 37°C for 1 hour. Plate 20-100 μL and incubate overnight at 37°C. Use a control plate with kanamycin and IPTG/X-Gal for observation.

II. GateV™ LR reaction:

1. Thaw the GateV™ LR Master Mix on ice and gently flick the tube several times to mix.
2. At room temperature, add the following reagents to a 1.5 mL centrifuge tube, mix gently, and briefly centrifuge in a centrifuge.

Entry clone （~50-100 ng）		1-7 ul
Expression Vector （150 ng/ ul）		1 ul
TE pH8.0	to	8 ul
GateV™ LR Master Mix		2 ul
Total:		10 ul

At the same time, perform the following controls:

pENTRY-LacZ （Entry clone）	1 ul
pLXTRC-403 (Expression vector，AmpR)	1 ul
TE pH8.0	6 ul
GateV™ LR Master Mix	2 ul
Total:	10 ul

3. Immediately return the GateV™ LR Master Mix to -80°C for long-term storage or to -20°C for short-term storage.
4. Incubate the centrifuge tube at 25°C for 1 hour. For large starting clones (→10 kb), incubate for 2 hours or overnight.
5. Add 1 μL of Proteinase K and mix well, incubate at 37°C for 10 minutes. Transformation: Take 1-5 μL of the reaction mixture from the centrifuge tube, add it to 50 μL of competent cells, incubate on ice for 20 minutes, heat shock at 42°C for 1 minute, return to ice for 2 minutes, add 250 μL of 2YT or SOC medium, and incubate at 37°C for 1 hour. Plate 20-100 μL and incubate overnight at 37°C. Use a control plate with ampicillin and IPTG/X-Gal for observation.

Notes:

1. The stability of the BP and LR Master Mix at room temperature is poor, so they must be stored long-term at -80°C to ensure their efficiency. -20°C is only for short-term storage, and can be stored for up to six months. Please use a -20°C refrigerator that does not automatically defrost. It is recommended to thaw in an ice bath and return to -80°C immediately after use.

2. For kanamycin-resistant vectors, the revival step can be omitted with little effect on transformation efficiency. For carbencillin-resistant vectors, revival must be performed or no transformants will be obtained.
3. Under optimized conditions of fragment and vector amounts and proportions, 1 ul of reaction mixture can generate 500-2000 transformants. However, for first-time users, it is recommended to use different volumes of reaction mixture for transformation, such as 1 ul, 2 ul, and 5 ul.
4. For controls, LacZ fragments are used, and to facilitate observation of recombination efficiency, it is recommended to use X-Gal and IPTG on plates.